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A STUDY OF THE RELATION BETWEEN FUNCTION AND GROWTH IN BODY CELLS.....*Montrose T. Burrows.*

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A Study of the Relation Between Function and Growth in Body Cells.

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IN THE BODY, growth is a definitely regulated act, which during development takes place irregularly in one part and then in another. At maturity it ceases in most tissues, except as it is necessary to replace tissue or cells lost by injury, to meet the demands of an increased function of the whole or a part, and to take care of the general wear and tear. Function, on the other hand, such as heart-muscle contraction, is something that, once established, goes on continuously throughout the life of the individual.

Growth in the body is, therefore, that which has a maturity or has limits, while function (rhythmical heart-muscle contraction) has none.

These two processes are related in only one regard: for a given amount of work of contraction there is a constant size. A stimulus which induces an increase in work on the part of the heart induces also an increase in the size of the organ, while one which induces a decrease in work leads to atrophy. What is true for the heart is also true for muscles in general and for the kidneys, liver and other organs of the body. Growth of the muscle fibers and cells of these organs is proportional, therefore, to their rate of activity or to the rate of their metabolism. That the same is true in the embryo, even before differentiation and the development of function, has been clearly pointed out by Child.* [1] Child again points out that with each decrease in growth rate there is a decrease in the rate of metabolism,

* Superior figures refer to numbered paragraphs of the bibliography at the end of this article.

and that this metabolic rate increases markedly in lower forms after starvation or when they are regenerating lost parts.

The question of growth regulation may, therefore, be temporarily set aside for a study of the conditions which regulate metabolism in general in the cell. As the tables for growth clearly show, the metabolic rate in the organism is never constant (Child and Thompson).[2] In early embryonic life the increment is high, then it gradually decreases, with irregularities, up to the death of the individual. In the early stages of development the blastomeres are simple, expanding, growing cells. Later they differentiate and assume a functional state. That this differentiation and the development of function is brought about by the continuous changing environment effected by the growth and filling up of cells in the growing individual has been clearly indicated by many already well-known studies of experimental embryology.[3] What we have not known are (1) the nature of those conditions which bring about these changes, and (2) the importance of these changes for the life of the organism itself.

In later life we have learned to recognize the fact, however, that all tissues do not become actively functioning cells. A part of the mesenchyme changes to muscle and glands, while another part remains in what appears to be a more primitive state, such as the cells of the interstitial tissue, bone and cartilage. The same is true for epithelium. A part is used for covering and protection and another part becomes functioning glands. For the sake of simplicity, I shall call the cells which are active in the glands, in the muscle, etc., as functioning cells, in contradistinction to those of the connective tissue, bone, cartilage and the epithelial coverings, the nonfunctioning cells. This is not a strict use of the terms, but the latter resemble more closely in their behavior the undifferentiated cells of early life.

There was a time when it was thought that all life processes centered within the cell. At the present time there is some question whether this is true. The primary changes in early development appear to be a rapid swelling of the mass, a rapid inbibition of water and oxygen, a rapid elimination of CO_2, and a secondary splitting of the whole into cells. Cellular growth, division and differentiation are not primary activities, but apparently secondary to other more formative forces or stimuli. Life does not manifest itself the same throughout the life of the animal. In the beginning it is recognized chiefly by the rapid expansion of the whole, the rapid division of cells and the careful building of its various parts. This period of building is completed in man, as is well known, within ten days after

birth. Subsequent to this time no new organ or parts of organs are formed except the laying down of the nerve sheaths. All subsequent growth is merely the expansion of previously formed structures. It is like hypertrophy and hyperplasia, as they result from stimulation of the adult organs.

With the completion of the building of the organs, function has made its appearance. Growth unlike that of the earlier period now runs hand in hand with new forms of work. This growth continues to maturity, when it ceases, except as it is to take care of the wear and tear, so to speak, and to play a part in certain organs and tissues, such as the bone marrow, the sex glands, and the nail- and hair-beds. Atrophy then slowly intervenes. This continues to the inevitable death.

The general nature of the structure and the metabolism which leads to this sequence of changes in the organism is the pertinent biological question to-day. Child[1] in 1915 reviews the general theories that have been advanced and attempts to give a physicochemical explanation of the process. Child, appreciating the relation between the slowing of growth and the decrease in metabolism, makes the general assumption, applying the "law of mass action," that the decrease in metabolism may be explained as the result of the gradual accumulation within the cell of an insoluble substratum. Since differentiation and the development of function takes place hand in hand with this slowing of metabolism, he looks upon them also in the same light. He considers the changes in the structure—differentiation—the result of the same slow deposit of the same substratum. This substratum he conceives as one of the products of the metabolic reaction. The reaction then, like any incomplete reversible physicochemical reaction, becomes slowed and this substratum accumulates, and ceases when it reaches a certain concentration. Death is the equilibrium point for this reaction.

While there is little doubt that many heterogeneous, like many homogeneous, reacting systems reach their equilibrium by this route, there does not seem to be sufficient evidence to show that the body is exactly of this kind. Again, all such systems which do arrive at such an equilibrium are first put together by some external force. In nature one cannot obtain more energy from any system than what has been put into it. *This is the law of the conservation of energy.* Child looks upon rejuvenation, then, as the result of the removal of this substratum and of dedifferentiation. Differentiation is not, therefore, according to Child, a change peculiar to a definite period, but

rather the result of growth, or the dynamic state of the cell. Senescence is an inevitable consequence suffered by all cells, whether they be unicellular organisms or cells of the metazoa. To put it in his own words, "Senescence is a necessary and inevitable feature of growth and differentiation, while rejuvenescence is associated with reduction." Differentiation he defines as a process of specialization. It takes place in the cells when they are suffering a moderately active metabolic rate. Decrease in this rate favors differentiation. Increase it and dedifferentiation tends to occur. Reduction is different. It is not the reverse of growth. Growth is the "accumulation of certain substances formed in the course of the reaction which are physiologically more stable than other substances that break down, furnish energy, and are eliminated." Reduction takes place when the breakdown is not balanced by the synthesis. Using Child's own data, I see no reason why this process may not be formulated, however, in a much more definite manner and in a manner more consistent with not only other natural phenomena, but also with the picture of development and later life as they have presented themselves through morphological studies. In the early periods of development, just after the fertilization of the egg, the rate of metabolism is high. The work performed during this period is the building of a heterogeneous mass out of a previous simple egg cell. This process of building is completed early. The metabolism at the time of completion is lower than at previous times, but it is still high. Subsequent to this time this metabolic rate, then, declines progressively to death. The picture at the beginning is therefore entirely different from the later one. It is the building of the machine, which, when once established, slowly runs down. The picture in development is that of the forcible putting together of parts, which are then to react with one another to produce work. This reaction continues in each case to an equilibrium or death, like any such machine built by man. While the metabolic rate is high in this machine it grows. This growth ceases (maturity) when this metabolic rate reaches a certain minimum. Then atrophy slowly sets in. Normal death is not the accumulation, but the gradual using up of the parts. It is the passing of heterogeneous system to a state of equilibrium.

Such an explanation looks upon the life of the organism, therefore, as the result of some unknown force, active only in its early period, beginning with the fertilization and ending with the formation of the last organ and parts of organs. This force disappears at

birth or thereabouts. Subsequent to this time life is merely an expression of the gradual deterioration of the previously built heterogeneous system or the interaction of its various parts, which follow the law for the disintegration of all such heterogeneous systems. Life of the animal ends with the establishment of an equilibrium between the parts of the organization which produce the work that really constitutes life. The energy for the building of the first period is derived, therefore, in the later period.

Systemic or *general death* is nothing more, therefore, than the establishment of a true equilibrium, or a breakdown in one of the essential parts of the body. This is not the end, however. What we have been talking about so far is the life of the organism, and not that of the cell. The end of the individual does not mean the end of all its parts. The cells are not dead at this period. They are all intact. This system is able to reproduce itself. The normal death of the whole is not the result of the death of the cells. The cells which make up the whole are destroyed, rather, by the process of the death of the whole. These cells, as we now know, go on forever under the proper circumstances. Out of one of them in the old, the egg cell, comes a new individual. Their destruction at the death of the individual is the result of this *general death*. Their destruction is the result alone of their position. *Systemic life* and *systemic death* are something different, therefore, from *elemental life* and *elemental death*. The first has limits; the other may have none. The question is, What is the nature of this system which can show such changes? This cannot be solved by a study of the animal as a whole. It cannot be solved by the study of the amœba, because it is an animal itself. It must be solved through a study of those cells which go through these changes, those cells which find this building of an animal and the ultimate disintegration of this animal their normal means for preserving their kind. The amœba need take no such complicated route to preserve its kind. These cells of the animal must take this course in order that they survive and that the proper environment be prepared for their reproduction. Child has ignored this fact completely. He has assumed a continuous dynamic state for the cell without any proof of its existence. He has again assumed that the life of the whole is only an elaboration of the life of the cell. He has assumed differentiation to be a peculiarity of all cellular life. He has assumed the protoplasm of cell to have structure capable of performing work. The best biological work of the last century has not only added no proof for

these general assumptions formulated by the earlier authors, but quite the reverse, it has spoken against them. As Bayliss states, there is not evidence that cells are necessarily dynamic. The best morphological studies of protoplasm have again failed to show structure in many cells other than nucleus and centrosome. As Wilson clearly states, cellular growth, division and differentiation are not primary factors in development, but secondary to more formative forces or stimuli. In the simple formulation which I have given above it is possible to understand how the organism not only reproduces itself, but it expresses definite need for the differential changes as they come into existence. The energy for the primary building is acquired from the old. The old is a machine not different in principle from other machines of nature.

As I shall show in the following pages, the main criticism of Child's theory is that he utilizes theories to build theories. He accepts the idea of the cell as the unit of manifested life of the organism without question. Before any theory of *life* and *death* is justifiable of acceptance it is necessary that the true nature of the structure and the metabolism of the cell be ascertained. This is not going to be accomplished by morphological and chemical methods alone, nor by a study of the metabolism of the whole, but by methods which allow us to study directly each of the fundamental manifestations of life, such as growth, division, differentiation, migratory movement, etc. The tissue culture has given us this opportunity. In support of the above contentions it is of interest to report here some general analyses so far carried out by this method.

THE CONNECTIVE-TISSUE CELLS.

As is well known, practically all previous work on the cell in regard to the nature of its energy-producing reactions and the manner of the transformation of this energy has been based upon the idea that the cell is a highly organized body. While for years it has been assumed that amœboid movements are comparable to surface tension changes in liquids, all theories have been based upon the idea that these amœboid movements are the result of localized changes in surface tension resulting from some unknown organization residing within the cell. All modern physiochemical methods have failed, however, to reveal any such organization. This has led one and then another to assume that the organization is either the result of a slow diffusion of substance in the colloids of the cell (Wells)[5], a peculiarity of colloids not yet organized, or to invisible membranes traversing the protoplasm.

As early as 1913[7] I had noted, however, that the movement of the cell of the organism is not one which is governed necessarily by such factors.[8] These cells in the medium of the cultures move always out and away from other cells; and in further studies carried on over several years, I have continually noted that the large number of these cells show no change in contour during their movement. Their movements are not amœboid. They glide along like bodies carried by some external force.

The picture observed in these cells is not that of a highly organized body, but one liberating a surface-tension-lowering substance. Their movements are like those of cayenne-pepper granules dropped on the surface of water. When a number of such granules are scattered on the surface of the water they shoot apart. This moving apart is the result of the liberation by them of a surface-tension-lowering substance. This accumulates in greater concentration between the granules. These granules are pulled apart, therefore, by the greater force of the water surface without.[9]

While the connective-tissue cells take the same course outward, they never become completely dispersed. These cells again fail to show movement on the surface or within a liquid medium, but move only and show evidence of metabolism in the presence of the fibrinogen contained in the blood plasma which I had used chiefly as a medium. In a liquid medium these cells round off to perfect spheres. In the plasma cultures they stick tightly to the fibrin formed in the coagulation of the plasma. In contact with these fibrils they spread out to take various shapes. These shapes are always peculiar to the surface of their contacts.[10]

Not only the character of the movement of these cells, but their general effect upon the clot, further indicates that they liberate such a surface-tension-lowering substance. This substance differs in its physical properties from that liberated by the granules of cayenne pepper, however, in that it is apparently not soluble in water, but it is adsorbed or chemically combined with the fibrinogen. The cells not only stick tightly to the fibrin, but they occasion its formation. When a fragment of connective tissue is placed in a drop of plasma it occasions first a gelation of the whole of the layer, and then later a true coagulation. This gelation commences at the tissue border and spreads rapidly, to invade quickly the whole of a large area of the fluid plasma. After a considerable latent period, the coagulation, the formation of fibrin and serum, commences. This true coagulation again begins at the tissue border and spreads slowly outwards, to

involve after many days a small area about the fragment.[11] With this second coagulation the cells appear. They are in close contact with the fibrin and they glide out just behind the spreading area of change in the jellylike clot. This movement continues only so long as the process of coagulation proceeds. The cells are elongated spindles closely cemented to the fibrin, yet capable of gliding on its surface. With the completion of the coagulation their movement ceases. They come to rest. In this state they will remain apparently indefinitely unless fresh fibrinogen is added. When this is added they again occasion its coagulation, move into it, and again come to rest when the coagulation is completed.

The whole picture of activity in these cells is that which can be readily interpreted in terms of the liberation by them of some substance insoluble in body fluids, but readily adsorbed or chemically combined with fibrinogen. The combination leads to the formation of fibrin. The movement and the clinging of the cell to the fibrin indicates further that this substance is one which has strong affinities for the cell. These cells do not crawl, but they glide, and are held firmly to the fibrin. Their gliding is directly proportional to the spread of the coagulant. Such can take place only in presence of a substance which is strongly attracted, not only by the fibrinogen, but also by the cell. Energy production in these cells is centered, therefore, about this substance. How it is formed in the cells becomes, then, a problem of interest. The fact that the cells come to what appears to be complete rest in the coagulated clot indicates that it is the product of the one reaction taking place within them.

For several years it has been evident that energy production in the body is the result of chemical change. It is further recognized, as Bayliss[6] clearly points out, that hydrogen and carbon enter chiefly into this reaction. Nitrogen in the body is used largely for the building of the substratum, proteins, in which these reactions proceed. This indicates that the reactions leading to protein syntheses are different from those producing energy.

What has not been shown is the nature of the products formed in this energy-producing reaction. It has been thought that oxygen enters directly into this reaction, and at one time it was thought that the products formed were largely carbon dioxide and water. Recent careful experiments have indicated that this is not true. Fletcher[12] has shown clearly that oxygen does not enter into the contraction phase of muscle, but is adsorbed largely in the recovery

period. Muscle may contract for a time without oxygen. J. Loeb[13] has found that oxygen is necessary to preserve the structure of the fertilized egg. These experiments of J. Loeb also indicate that the metabolic reaction may proceed without oxygen. That the same is true for the animal cells has long been known by pathologists. Cut off the blood supply from any part and it undergoes a coagulative necrosis. These observations suggest strongly, therefore, that oxygen plays a necessary but probably a secondary rôle in this reaction. It acts to remove certain products of the primary reaction rather than enter into it.

All chemical reactions, as it is now fully appreciated, are governed not only by the concentration of reacting substances, but also by the concentration of one or all of the products formed. Equilibrium for a simple reversible reaction is expressed in the following formula:

$$A + B + C + \ldots = A' + B' + C' + \ldots$$

In the animal organism growth is not determined by food any more than it is determined by oxygen, but by other unknown factors. Bardeen showed that planaria regenerate their parts when starved as when they are fed.[14] Morgan found that salamanders regenerate their legs as rapidly when starved as when fed.[15] The difference is that the starved animals suffer marked emaciation and a general atrophy of their organs.

The above observations of the connective-tissue cells indicates clearly the existence of a previously unrecognized product of cell metabolism which is evidently insoluble in liquid medium, but rapidly adsorbed and chemically combined with fibrinogen. This substance, which I shall designate as "L," is also an active blood coagulant. It is something, therefore, which is insoluble in water, but capable of combining with fibrinogen to form an insoluble combination, fibrin. It acts in every regard, therefore, like the lipoid fraction of the blood coagulant recognized by Woolbridge[16] and recently carefully studied by Mills.[17] This fraction is a phospholipin or a group of such bodies.

In a recent series of experiments[9] I undertook, therefore, to ascertain more carefully the exact relation to this substance of the general chemical changes of these cells. These cells had apparently become inactive after they had come to rest in the clotted fibrin. They had laid for months in this inactive state and in the presence of oxygen. When removed to fresh medium they had again become actively migrating cells. I removed the oxygen from about these cells during their movement and also after they had come to rest.

While they are migrating and the coagulation is taking place they disintegrate in the absence of oxygen. They suffer a coagulative necrosis. After they come to rest they suffer no change in the absence of gas. In the same way, these cells suspended in a liquid are unaffected by the absence of oxygen. In a liquid they do not metabolize any more than in the presence of a fully formed fibrin. Any slight stimulus which occasions their movement occasions also their disintegration in an atmosphere of nitrogen.

There seemed little doubt, therefore, that in the identification of this "L" substance the regulator of the metabolism of these cells had been found. It is not something which is continuously washed away by the blood stream, but, quite to the contrary, it has very specific affinities, and when brought into contact with fibrinogen it forms an insoluble fibrin. This suggested strongly, therefore, that continuous activity in these cells must depend not alone on food and oxygen, but also upon very special conditions for removing these primary products from the cell.[9] The only plentiful substance which I have been able to find in the adult body capable of such removal is fibrinogen. This is transferred by the "L" substance into fibrin. In the body the messenchyme cells of older embryos and the connective-tissue cells of adults lay down extracellular fibrils. Hertzler[18] has shown very definitely that fibrin forms the basic proteins of these extracellular or collagen fibrils.[19] These results of Hertzler have been confirmed by Baitsell[20] and myself.[11]

These observations indicate that the connective-tissue cells of the body are not in any sense continuously active. Their only function is the production of extracellular fibrils. They do not secrete these fibers, but coagulate certain proteins formed elsewhere in the organism. The increase in these fibrils is alone indicative of an active metabolism in them. The dynamic state of the organism is in no sense, therefore, an elaboration of the dynamic state of these cells. It must depend on other conditions. This led me to investigate more carefully the process of rhythmical contraction as it is seen in heart-muscle cells.

In my earlier studies of the tissue culture I had already shown that differentiation in heart muscle is a purely reversible phenomenon.[21] The actively contracting heart-muscle cells are derived primarily from the undifferentiated mesenchyme. When the fragments of this tissue are brought into contact with the plasmatic medium of the culture the heart-muscle cells at the edge of the fragment which had been contracting lose this property at once. They migrate into

the medium and behave exactly like the simple undifferentiated mesenchyme cells. In 1912 I [21] showed, however, that in a few cases these cells which migrate into the medium may differentiate again and develop rhythm. When this takes place it is interesting to note that the cells by chance alone had come into a very peculiar relation with the medium, and again it is interesting to note that this rhythm never develops while the cells are migrating, but always after such migration has ceased and the coagulation process is complete.

The differentiation of the heart-muscle cells in the outer medium takes place very infrequently. In the large majority of the culture of the older embryos and adults the clots cling tightly to the fragment. The cells migrate in contact with the surface of the fibrin fibrils. When differentiation takes place the process is different. The clot loosens and contracts away in mass. If the ends of a few cells remain attached to this clot they may become stretched through the serum cavity between the surface of the medium and the end of the fibrin fibrils, or between the fragments and the end of these fibrils. After the coagulation is complete, these cells, and these cells alone, develop rhythm. If they be removed from those contacts and be placed in the outer medium in contact with the surface of the fibrin they stretch out again and behave like simple mesenchyme or connective tissue cells.[9]

By these observations it became possible, therefore, to clear up the difficulty of the earlier observations of the connective-tissue cells. Dynamic states in the organism, such as the heartbeat, are not a property of the cell, but that of a peculiar organization of the environment. These cells may produce the energy for the work of the body, but there is no evidence that the transformation of this energy into work is the product of a cell organization in the case of the heartbeat any more than it had been found to be in the case of cellular migration.

What is true for the connective-tissue cells I find also to be true for the epithelial cells of the skin and many of the glands. The gland cells in the cultures lose the form peculiar to them in the organism. They stretch out, like the skin epithelium, to form broad, thin sheets of cells. These cells cannot metabolize except in the presence of fibrinogen or a similar adsorbing substance. They differ from the connective tissue in that they later destroy this substance through certain added proteolytic properties. They thus depend wholly on the fibrinogen for their activity in the culture, but they remain together and form no extracellular fibrils, in that they later

destroy the fibrin which is formed.[23] In liquid media they show no evident activity.

While these experiments are interesting for the understanding of the general problems of the genesis of connective tissue, their broader significance lies in the new view they present of life in the organism. They show that the continuous activity consistent with life is not a property of these nonfunctioning cells. There must be long periods of time when the connective-tissue cells show no activity. Life must be, therefore, wholly a part of the functioning systems, the glands, the muscles, the brains, etc. In early life, however, this cannot be true. The whole problem of life in the early embryo is centered about an excessive metabolism and an active proliferation of the undifferentiated cells of this earlier period.

Many years ago Hofmeister, Sachs and De Bary had already shown that cellular proliferation is not a primary factor in the growth of plants. They thought that the mass forms in growing plants before it breaks into cells. That the same is true for animals has been clearly enunciated by Whitman, Adam Sedgwick, E. B. Wilson and others. In development, cellular growth, division and differentiation are not primary factors, but they are again secondary to other more formative forces. Driesch looked upon this force as something apart from nature which cannot be solved. Others have not looked at it in this sense. They have considered that this early development is the result of certain reactions which occasion such a primary building. Many general physiologists have attempted to arrive at its solution through the study of colloidal swelling. It is evident, however, that such study cannot attain directly to this goal. At the best they can only indicate analogies. It occurred to me to attempt the solution by another method. The above studies were made with connective-tissue cells of adults and the mesenchyme cells and heart-muscle cells of older embryos. No careful comparison had been made of these cells with those of the younger embryos. In the younger embryos it is well known that the mesenchyme cells form no extracellular fibrils, but grow actively to form a cellular syncytium. The fibrils appear only in later embryonic and adult life.

THE BEHAVIOR OF THE CELLS OF THE YOUNGER EMBRYOS.

As I have cited in a previous article, M. R. and W. H. Lewis, in 1911,[24] noted an active movement and growth of cells in liquid medium such as simple salt solution. This, as I have just stated, is not true of the adult connective-tissue cells. Harrison[25] in analyzing

this movement noted that the sells never moved directly outwards into the liquid but always at its surfaces. In Harrison's earlier studies he had attempted to cultivate fragments of the neural tube of frog embryos in hanging drops of serum. No evident activity was observed. Success was attained only when these fragments were placed in lymph which clotted about them. In studying the movement of the cells in lymph Harrison then noted that the cells moved always in contact with the fibrin fibrils, and in his later analysis of the culture of the Lewises he found the cells migrating always in contact with the surface of the cover glass or on the free surface of the medium. Harrison[26] termed this phenomenon, as L. Loeb[27] had done, "stereotropism." He considered these cells apparently highly complex systems whose mechanism for movement is regulated through such contacts. Both authors thought this a common property of all cells.

After a careful study of the movement of these embryonic cells in liquid medium, I noted, however, that these cultures of the Lewises were applicable only for the movement and growth of the cells of the younger embryos. No activity is seen about the normal fragments of heart muscle or other mesenchyme cells of older embryos at the surface of the liquid or in contact with any solid. As I have stated above, blood plasma or fibrinogen is the one common substance of the body capable of stimulating activity in them.

From these facts it seems evident, therefore, that it is not solids in a general sense that are necessary for the movement of these cells, but specific adsorbing substances. The younger embryonic cells differ from the older ones in that they may move at the surface of the salt solution and liquid media as well as in the clot. The older cells had lost this property. This leads me then to analyze more carefully this movement at the surface of the medium. I measured the position of the cells moving at the cover-glass surface with the micrometer of the microscope. I found that these cells were not in contact with the cover glass, as Harrison, Lewis[28] and L. Loeb had thought, but that they lay often a considerable and measurable distance below it. Again, previous to the movement of the cells on the surfaces of the hanging drop, these surfaces change. They became covered by a film or scum, which made them appear leathery. The cells in every case moved, grew and divided in this film of material. This substance is not the coagulating substance "L" liberated by the other cells. It is something new, which acts evidently in place of and antagonistically to

the fibrinogen. This substance formed early in the life of the culture. It spreads rapidly to cover the whole of the medium. The cells later invade it as they invade the plasma. It differs from the fibrinogen in that it is a much more active stimulant. The cells form no insoluble compound with it. They grow actively for a time within it and invariably disintegrate. In the plasma cultures of these younger cells, a part of the cells may at first invade the clot. They soon leave the fibrinogen, however, for the surface film which has stronger affinities for them. Single cells may also liberate this substance.

While I do not know the exact nature of this substance, it is interesting to note here that this substance disappears from or its action is inhibited about fragments of undifferentiated mesenchyme and the heart-muscle tissue in the cultures between the ninth and fourteenth day of the incubator life of the chick embryo. This is not true, however, of other tissue. I have identified it in the epidermis and the liver of these embryos as late as the eighteenth day. Felix has shown the new tubules developed in the kidney of man as late as ten days after birth. While these studies are yet incomplete, they do indicate that the disappearance or the inhibition of the action of this substance is not regular, but takes place at irregular times in different tissue of these higher animals, and that this disappearance corresponds to the forms taken by the developing embryo and adult.

What might be the exact chemistry of this substance remains to be seen. It seems most plausible, however, that the secrets of the building of the body will be found in the physical properties of it. These physical properties are, first, a strong affinity for water, and, second, an ability to stimulate an excessive metabolism.

From the above observation it became evident, therefore, that the cells of the body are not in a continuous state of activity. The activity of early life is dependent completely upon the synthesis of a substance which removes the "L" substance from the cells. This substance is gradually superseded in later life by fibrinogen. Fibrinogen combines with "L" to form fibrin, an insoluble compound. In the later periods life centers about the functioning or differentiated systems. To prove this fact more definitely it became of interest to study the effect of a flowing stream of serum upon rhythmically contracting cells and the dedifferentiated muscle cells and the cells of connective tissue.

THE CONTRACTING HEART MUSCLE CELLS.

The isolated contracting heart-muscle cells which occasionally develop in these cultures from cells migrating from the fragments contract with a rhythm like that of the whole hearts or fragments of the heart transferred to the cultures. Since they occur most infrequently, contracting fragments have been used chiefly for this study.

The rhythm of the fragments and the whole hearts of younger embryos may never cease when transferred from the chick embryo to the medium. If it does it commences at once again as soon as the temperature for it is restored. During the first few minutes or hours this rhythm is regular; and in the case of the whole hearts and fragments of the ventricle, it is the same as that which occurs normally in the body. After a short time, however, it becomes irregular. There are periods of activity followed by periods of complete rest. These periods of activity, as I have previously described them, are ushered in by rapid, strong contractions. These contractions gradually decrease in amplitude and rate to the period of complete rest. After a short rest period the active rhythm again intervenes, and so on; their irregular rhythm may continue for as long as eight or nine days in a single hanging drop.

This slowing of the rhythm and rest, I concluded, was due to the temporary accumulation of waste products, the temporary lack of nutrient substances, or both. After these waste products had slowly diffused away and nutrient substances had moved in, the heart became active again. To prove this I placed several such fragments in a specially devised culture chamber. This chamber was arranged so that serum could be made to flow continuously along a cotton wick, the fibers of which transversed the layer of plasmatic medium. By this means it was possible to continuously wash the medium about the fragment. This culture has been called the "wick culture."[29] In such cultures the rhythm of the fragments remains regular during the time the serum is flowing, and this regular rhythm continues often for many days or until the protoplasm of the cells is otherwise destroyed by infection, etc. A careful comparison of the irregularities in the case of the contracting heart-muscle cells with those of the migrating ones shows interesting differences. In the case of the migrating and growing cells there are no intermittent rest periods. Migration commences after a latent period. It continues actively for a time; then gradually ceases. In the same culture there is no second recovery period.

Again it is interesting to note that the changes in the clot are different in the case of these two activities. The migrating cells liberate a substance which occasions a coagulation of the clot. About the contracting fragments from which no cells are growing no such changes are seen in the clot. Contraction in the isolated cells commences after they have ceased to migrate and show these changes.

The substance or substances liberated by the heart which prevents its contraction and which are evidently concerned with this act of contraction are soluble substances. They can be washed away with serum. The evidence gleaned from the above studies on the migrating cells indicates that the substance or substances which occasioned their migration are insoluble in the medium. They are adsorbed or chemically combined with the fibrinogen to form fibrin. The substances which accumulated to stop the activity of the heart is not the "L" substance noted above.

For the testing of the connective tissue and undifferentiated heart-muscle cells I have used a glass culture chamber (plate LII) instead of the one described in the previous paper.[29] Otherwise the technic was the same.

During the course of the study of the contracting heart-muscle cells in 1912 I had thought that the migrating cells move more rapidly against the stream and that they became more dispersed in these cultures than in the simple hanging drops of medium. These differences were observed, however, in but a few cultures. Later I noticed that the thickness of the layers of medium also affected the movement of the cells. The cells migrating from 1 mm. thick fragments, which had been placed near the edge of the hanging drop, moved more actively in the thin edge[7] than in the thicker parts of the layer within.[8] Regulating these conditions in the "wick cultures," I found that the flowing serum in no way affected the migrating heart-muscle cells, nor did it effect in any way the movement of the epithelial and connective-tissue cells in general. The only cells affected were the leucocytes and lymphocytes. In the simple hanging-drop cultures it takes several transplants to effect a complete removal of the leucocytes and lymphocytes from fragments of bone marrow, lymph gland and spleen. In these "wick cultures" I found fragments of spleen entirely stripped of these cells after seventy-two hours. The leucocytes and lymphocytes had accumulated in masses at tangled parts of the wick.

The conditions which regulate the movement of these latter cells

are evidently different from those which control the movements of the fixed tissue cells of the body. Migratory movements and growth of the heart-muscle cells are effected by a substance which is insoluble in circulating body fluids. Growth in these cells is therefore a purely physico-chemical reaction which proceeds to a condition of static equilibrium. For growth to take place it is necessary that this substance or these substances be removed. As they increase all activity ceases. This substance or substances I have designated as "L."

DISCUSSION AND CONCLUSIONS.

From these observations there seemed little doubt, therefore, that the dynamic state of the organism is not in any sense an indication of a similar dynamic state in the cell. It is a product of the organization of the body. In early life the dynamic state is associated with the formation of a substance or substances which combine with or otherwise remove the "L" substance of the cell and occasion an active metabolism within them. This substance has strong affinities for water. It is thus directly concerned with the early building of the organism. It stimulates not only an excessive metabolism in these cells, but also may occasion the primary swelling of the mass. This substance disappears, or rather it ceases to be recognizable, in the early period of development. Subsequent to this period, life becomes subservient to new organizations. The new organizations, the general nature of which I have illustrated in the study of the rhythmical contractions of muscle cells, are not unique, however, for the tissue. They are peculiar to all functioning cells. The nerve fibers are stretched between the brain and an end organ. Adrian has shown that the "*all* or *nothing*" law holds for this tissue like it does for the heart. The same is true for the glands. The secretory cells are cells which have a free end and one attached to a basement membrane. Stop up the ducts of one of these glands and these cells undergo atrophy. Such observations are wholly in line with the general facts which are known concerning development. In man the kidney may form tubules up to the tenth day after birth. Other organs cease their progressive growth much earlier. No careful studies are known concerning the time the heart cells cease to divide. In the later embryonic period, at least, all growth in the heart is represented by an increase in the size of the fibers rather than an increase in the cells. In the kidneys and glands it manifests itself in a dilation and increase in length of tubules and a flattening of their

lining cells. In the wound the exudate is the active stimulus for growth. It stimulates metabolism through a direct affinity of the "L" substance for the fibrinogen. "L" combines with fibrinogen to form fibrin. The fibrin then becomes the extracellular or collagen fibrils. The cells remain active and move towards the center of the exudate until the reaction is satisfied. The end of activity is the completion of the reaction. It is the scar for the connective tissue and continuity, and the limiting membrane for the epithelial cells.

Through the analysis of the simple act of changes of shape and the movement of the connective-tissue cells it has been possible to show that these acts are not the result of any complex cellular organization, but a simple reaction between substances in the environment and a substance of the cell. The movement is the result of the cohesion of this substance for the cell on one side and specific substances in the medium on the other. In this act of locomotion the cell supplies the energy; the mechanism otherwise resides wholly in the environment. Again, in other studies I have been able to show that the syntheses for growth are not a part of these energy-producing reactions, but they are separate reactions. The connective-tissue cells in the adult organism are widely separated in a mass of fibrillar substance. These cells migrate into the plasma, but they do not grow. In the plasma culture, growth is peculiar alone to the more cellular fragments of embryonic tissue, granulation tissue or sarcomata. By the use of embryonic extracts it is possible to stimulate the metabolism of these connective-tissue cells. Under these conditions the widely separated cells of the adult fragments will grow and divide. About the more densely cellular fragments such stimulation is not necessary. It is harmful. It leads to the destruction of the cell, a breaking down of the proteins of the protoplasm. If these same fragments are teased apart so the cells become more dispersed, growth ceases again. For growth to take place it is necessary that the cells be either crowded or excessively stimulated. The important factor for this reaction is evidently the concentration of certain products of their metabolism. The concentrating of such products may be induced by the crowding of cells or increasing the rate of their production. These cells liberate not only the "L" substance, but also CO_2 and H_2O. How many other substances are formed when the reaction is proceeding has not been determined. Protein synthesis is not a part of the ordinary metabolic reaction of the cell. It is secondary reaction depending upon and obtaining the

energy necessary for it from the energy-producing reaction of the cell. The extracellular fibers of the connective tissue are not secretions of the cells any more than bone or cartilage are of this origin.[5] These fibers are the combination of proteins formed elsewhere in the body and the "L" substance of these cells.

There is no reason to believe, therefore, that protein synthesis is a part of the metabolism of the cell. It is something different. It is a form of work produced. Upon it growth depends. Growth has never been shown to be of a simple chemical or physical nature. It is the result of the careful utilization of energy. It takes place against the forces of nature. Protein synthesis, like muscular contraction, is only a form of work peculiar to body organizations and not cellular organizations.

In 1917,[30] and again in recent experiments,[9] I have shown that the development of ryhthmical contraction is not associated with any fundamental change in the cell. This is a property peculiar to any of the mesenchyme cells of early embryonic life. It occurs in the fragments of this tissue and in the cells which migrate from them. Its development is the result of a chance relationship of the cells to medium. This relation is wholly dependent upon the physical peculiarities of the coagulation of the plasma clot. The isolated cells which develop rhythmical contraction are those cells which become stretched through a serum cavity between the surface of the medium or a cellular tissue fragment and the end of fully formed fibrin fibrils. No contractions develop until these fibrin fibrils are fully formed. What is true for the isolated cell is also true for the fragments. Fragments of the heart of young embryos contract at once when removed to warm medium of the culture. Those from older embryos fail to show this change. Rhythm develops in these latter fragments only after the border cells have moved out or the process of coagulation is completed. The end of the cell in contact with the fibrin is in metabolic equilibrium. These cells imbedded in fully formed clot cease all activity. The cells floating in serum also show no change. The only active part of these cells differentiated for contraction is the free end in contact with the cellular fragment or the surface of the medium. This end suffers a decrease in surface tension. Such a decrease in surface tension is associated with electrical changes at this end of the cell and a stretching of the cell. If such changes continue, one of three conditions must result: the cell will be torn loose or in two, or there will be an explosive breakdown of this

surface-tension-lowering substance. That such an explosive breakdown is peculiar to the contraction is clearly indicated by correlating these observations with those of Fletcher. Fletcher has found that lactic acid is liberated during the contraction phase of muscle. I find that lactic acid increases the surface tension of the cells. It causes the cell to contract. This lactic acid disappears again during the intermittent rest period. The cell again returns to its former state. The process is rhythmical. The process, as it is evident, is somewhat similar, therefore, to the phenomenon described by Bredig. Bredig showed that when a ten per cent solution of H_2O_2 is placed over the surface of pure mercury, a film of mercury peroxide forms at the surface. This leads to electrical changes in this system. Under appropriate conditions the peroxidate breaks down again to mercury and oxygen. Then the layer of peroxidate reforms. The process is repeatable or rhythmical. Bredig and his students further find that many conditions which alter rhythmical muscular contraction also alters the activity of this model.[31]

Rhythmical muscular contraction is not, therefore, as Bayliss suggests, the result of rhythmical stimuli, but evidently the result of an explosive breakdown leading to alternate changes, not only in surface tension, but in the electrical conditions at opposite ends of the cell. Bernstein[32] several years ago had given definite evidence to show that the energy of muscular contraction is surface energy. He found that muscles suffer the thermal changes peculiar alone to surface energy.

In such organization, as it is well recognized, there is no reason that such an explosive breakdown should always occur. Such is possible only when the decrease in surface tension at the free end or the electrical changes are of such a degree to allow a current to pass through the resistent cell. This change at the free end may cease before such is possible. For a breakdown to take place other special conditions or stimuli must be present. That this is the condition of most of the functioning tissue of the body has been well proven by the studies of tissue autonomy. In man, whether any muscular tissue, other than the nodes of the auricle, are autonomous, like the cell of the culture, I think is questionable. At the same time it is evident that any functioning tissue may develop such a condition. Whether this is what has happened in many of the nervous affections might be a problem worth investigating. It is not surprising, however, that in other animals the autonomy is centered in other tissues. Carlson[33] finds that the rhythm of the heart of the limulus is not automatic, but centered in certain

ganglion cells. In men I think it is very questionable whether the nervous system, any more than the striated muscle or gland cells, respond without the aid of external conditions. It is upon this fact that coördination depends.

There is no evidence from the above observations that the cells themselves undergo any absolute changes during these fundamental changes in the body. For a good many years it has been well known that bone and cartilage are products of a given organization of the part and not a product of the cell. It has been shown, for instance, that bone will develop in the pelvis of the kidney if the blood vessels to that organ are ligated. Asami and W. Dock[34] repeated these experiments in the laboratory and proved completely the existence of these changes, as the other authors had shown. In the tissue culture the cells migrate readily, not only from fragments of interstitial tissue, tendons and fascia, but also from fragments of bone and cartilage. The bone cells and cartilage cells behave in every way like the other connective-tissue cells. The bone and cartilage remains behind like the extracellular fibrils. In the same way the adult muscle fiber will not react in the plasma, but I have seen the nucleus and sarcoplasm migrate out and leave the adult fiber behind. In the plasma this mass forms perfect connective-tissue cells. These cells react also in every way like the ordinary connective-tissue cells.

Such simple fluid systems may not only suffer changes in shape and differential changes of tension at various points on their surface, but they may also suffer additions and probably subtractions from themselves. In the body differentiation is not only the result of mechanical changes initiated and controlled by the environment, but it may be also chemical in nature. As the above studies of the skin epithelium show, these cells do not suffer fundamentally from the connective-tissue cells. For metabolism to take place within them it is necessary that the "L" substance be removed from them. This "L" substance is not different in these cells from that of the connective-tissue cells. These cells differ from the connective tissue in that they also contain a proteolytic ferment. The pancreas cells again differ in that they also contain a fat-splitting ferment. They often fail to migrate into the clot. They lead rather to the rapid splitting of the fat of the plasma to fatty acid crystals. This prevents also the migration of the connective-tissue cells from these fragments into the medium. So in each case the various tissues differ as they contain their own peculiar added products. The fun-

damental reaction of the fixed tissues otherwise remains the same. The only exception to this rule is to be found in the wandering cells. These cells have not been observed to grow in the cultures. They have also lost their ability to cause a true coagulation of the plasma. They occasion the gelation of the clot and can move only in contact with this jellylike mass, but they cause no fibrin formation. They owe their spherical shape to their inability to form true surfaces in the medium. They move by a mechanism different from the fixed tissue. They can invade these fixed tissues in the presence, at least, of an exudate. When this disappears they tend to move back into the lymphatics and blood capillaries. They do not repel strongly the moving fixed tissues like the fixed tissues repel each other. They have lost the property to form the "L" substances. I say they have lost it because it is present in the mother cells from which the wandering cells arise.

To what extent this chemical differentiation is reversible like that peculiar to the mechanical form I have not definitely determined. There is evidence to show that the mesenchyme cells may arise from epithelial cells even in late embryonic life, but for the most part these epithelial cells maintain their chemical peculiarities for a long time in cancerous growths and in the cultures. I have seen heart-muscle cells assume the characteristics of large mononuclear cells. The reverse has not, however, so far been proven. Again I have seen liver cells, after repeated transplantation, behave in part at least like the connective-tissue cells. There is no reason to believe, however, that such chemical dedifferentiation may not occur and maintain in the proper environment.

The cells of the organism are not, therefore, highly complex systems. They are not equipped to lead an independent existence. They do not age. They have no organization for work. They produce the energy, but the work or their various manifestations of life is dependent wholly upon external conditions about them. The only form of work depending on an evident internal organization is cell division. The forces active in the process center about the centrosomes. There is no evidence, however, that activation of these centers, or even their formation, is controlled from within. The centrosomes develop probably alone in response to external stimuli (see Hertwig, Meade, Morgan, J. Loeb, E. B. Wilson[3] and others).

Energy production in these cells is again wholly dependent upon organization, or the presence of specific substances which split or otherwise make the removal of the "L" substances possible. Their

activity in early life is dependent upon the synthesis of a substance which combines or otherwise removes the "L" substance. In later life it is dependent upon a mechanical differentiation. This form of synthesis in later embryonic life forms a substance which is fibrinogen or closely akin it. This forms an insoluble compound with "L" rather than the soaplike substance of the earlier period. In the proper environment it occasions, through its coagulation, the form necessary for the dynamic state.

By these observations it has been possible, therefore, for the first time to define differentiation in other than morphological terms, and to compare function with growth. The growth of the undifferentiated cells of early life is the result of a special synthesis. The development of function is the result of a slightly different one. Differentiation is quite different, therefore, from what it has been conceived to be.

While these observations reduce growth and function to simple physicochemical formulation, they give no hint as to the cause for this change in synthesis from early to late life. Any substance which removes the "L" causes an immediate loss of mechanical differentiation. The heart-muscle cells in contact with the fibrin become simple mesenchyme cells. Only under proper mechanical conditions can they redifferentiate. What is true for the heart is true for the glands. In the cultures the gland cells stretch to form membrane like the skin. They lose the form necessary for function.

These facts, again, do not explain chemical differentiation. In the above observation I have also not discussed all the deficiency of these cells. Besides the lack of any organization for work and any means within themselves to allow the energy-producing reaction to proceed under the ordinary conditions in nature, they are also bereft of the property of using the crude material of nature for this energy-producing reaction. While energy production in the body is derived chiefly from H and C, these cells cannot use the sugar carried to them by the blood stream without intervention of substances from the pancreas. The organization of the whole or certain of its parts are again essential for another of their important needs.

It is upon this last deficiency that the chemical syntheses peculiar to differentiation must depend. The body is a machine operated like any other machine in nature. Its metabolism has no complexities, as most biologists would have it. It is a machine which is able to produce energy and transform it into work. It has been possible to locate these different reactions. The energy is produced in the cell;

its transformation is under the control of the environment. Protein synthesis is in no way to be confused with the energy-producing reaction. In the body, anything which increases metabolism will lead to growth. Increase this still more and protein destruction results. This accounts for the appearance of split products of protein in muscle which has suffered excessive stimulation (see Bayliss for literature) and the destruction of the tissue by strong growth stimuli such as X-ray, radium, coal tar, arsenic, etc.

Such a machine can build itself in the manner that it does because it utilizes the products of its energy-producing reaction for its building. The building is only the sequence of change its original fundamental structure undergoes to produce the final necessary work—the work of supplying an adequate amount of fuel for a certain period of life and supplying the egg with an adequate supply of this material (yolk) to carry it through the early period of the development of the whole. This yolk supply decreases progressively. This decrease leads to the changing character of the syntheses.

The extracellular deposits which I have described above as important for the organization peculiar to the dynamic state of later life are evidently the substratum recognized by Child. They are the result of protein syntheses peculiar to the organization of a certain period of the development of the whole. They must be the result not only of mechanical but also chemical changes in the environment. The picture of the organization as it is seen through the study of the cells is in no sense the picture of one continuous metabolic change. It is the primary building of a heterogenous system followed by the gradual decline of this system to a state of static equilibrium. *Elemental life* and *elemental death* are not comparable to *systemic life* and *systemic death*. The body is the necessary cycle that these cells may preserve their kind. For the active growth of the early period of the development the cell draws upon the yolk or the mother for its supply of those substances necessary for this growth. The disappearance for this supply of material is the appearance of the second, or the functioning, period. The building of this period is completed within ten days after birth in man, except for the laying down of the nerve sheath. The syntheses peculiar for this building are the result of the disappearance of the substances carried in the yolk. The important factors for differentiation are not to be found, therefore, in the primary reactions of the cells, but in the deficiencies of these cells. The important deficiency which

has to do with differentiation is their inability to utilize the crude food materials supplied by nature. This deduction we are forced to believe, however, not only from the above observations, but from a host of already carefully accumulated facts. If the first two blastomeres of many lower forms are separated, two animals develop. The yolk is also separated by this process. Each animal is but one-half the size. Gudernatsch has shown that tadpoles fed upon thyroid differentiate within a few days, while thymus feeding delays this process at least for a very long time. Very large tadpoles may be thus developed. The ultimate heterogenicity thus developed is essential for subsequent life and for reproduction. The egg cell builds a new system like the old (heredity), in that it has acquired from the old a supply of those substances necessary to carry it through the building of the new system, which is again capable of preparing these substances from the crude materials available in nature without. The building results from the changing syntheses which result from the gradual decreasing yolk supply. The form of the building is dependent, therefore, primarily on the original constitution of the yolk. This is again dependent absolutely on the nature of the machine which produces it. Each animal must, therefore, in each case reproduce its kind. The problem of heredity is thus reduced to pure physics and chemistry.

Death in such a system may result, therefore, from the destruction of essential parts, or the inevitable equilibrium of those forces which maintain the heterogenicity. This does not mean a fundamental change in the cell. The cell succumbs as the result of this breakdown. There is no reason why any of these cells may not grow actively again if the organization is changed about it. Differentiation is both chemical and mechanical in nature. Chemical differentiation does not effect the fundamental energy-producing reaction of the fixed tissue cells. It is an indication of the chemical heterogenicity of the mature organism. The mechanical differentiation essential for the dynamic state is reversible. According to the above formulation, mechanical differentiation in early life is the result of the removal and decrease in certain materials of the yolk. If a change in organization suitable for active growth should take place in the organism, no such differentiation should follow. The cells suffering such changes must continue to grow as long as the body supplies the necessary substances. The body must supply those substances necessary for active growth as long as it survives.

A rapid utilization of these substances must lead to a rapid atrophy and death, according, also, to the formulation given above.

Such a reorganization, it is evident, cannot take place in early life except under the influence of most powerful external stimuli or developmental defects. It becomes, however, more possible in later life when the normal forces which maintain the normal heterogenicity are waning. Cancer represents such a form of active growth. It is a disease peculiar to later life. It occurs earlier in connective-tissue areas than in epithelial tissue. The connective-tissue cells, according to the above observations, lose their property for independent growth earlier than the epithelial tissue.

In previous publications[9] I have noted that cancerous tissue grows like that of younger embryos and liberates the same or a similar growth stimulus.[35] In the body cancer may result from congenital abnormalities, such as pigmented moles. It occurs more frequently in certain families of mice (Maude Slye). It follows the continuous application of many growth stimuli or substances capable of effecting such a reorganization. In man, as pathologists agree, it follows most frequently upon long-standing chronic inflammation (Billroth).[36]

As has long been fully appreciated, the impediment which has stood in the way of advance in cancer has been the inadequacy of our knowledge of the cell and its relation to the whole. It has been the endeavor of the cancer laboratory in St. Louis to attack the problem from this route. Cancer is not a parasitic disease. It is a disease which follows after long, continuous stimulation. Before cancer can be understood it is necessary that irritability and stimulation be reduced to simple terms. The essential conditions for mechanical dedifferentiation, or loss of function, and the production of an active growth of cells like that of cancer is the presence of substance capable of removing the "L." This "L" has many of the properties of the phospholipins isolated by Mills. These phospholipins are soluble in many lipoid solvents, and especially products of coal tar. We have studied the action of coal tar, and find that it behaves in the tissue like the substance liberated by the cancerous tissue and the tissue of young embryo which is able to combine with or otherwise remove the "L" from the cells. Coal tar thus attracts the cells to it and effects their dedifferentiation. At first it occasions their disintegration. Later it becomes a less active solvent and occasions an active proliferation of these cells. It is capable not only of producing many of the symptoms of cancer by itself, but of

effecting the reorganization necessary for true cancer to develop. Long use of coal tar leads to the development of cancer.[35]

What I have hoped to present in this paper is not only the general picture of the cell the tissue culture has so far revealed, but methods by which the further details of these general problems may be attacked. From the above observations it is evident that the problems relative to life are not to be solved by chemical and morphological methods, but by the application of mechanism. Heredity, as we have seen, is dependent wholly on the chemistry of the system, but the importance of these chemical substances will not be understood until the mechanisms peculiar for the various manifestations of life have been isolated, their parts and the energy relation between these parts have been fully determined. Upon this latter knowledge rests also the ultimate control of cancer. Cancer, as it has been seen, does not arise from embryonal cells. It is not the mere displacement of cell (Ribbert), but a specific reorganization of parts. Such a reorganization means a breaking down of the normal relation of cells in the adult. The normal heterogenicity is maintained through the fact that each kind of cell moves through the liberation of the same surface-tension-lowering substance. Each tissue repels its neighbor like each cell repels each other cell of the same or of another kind. It is a breaking down of this barrier through the synthesis of a new substance which conditions new surface relations. It is an invasion of connective tissue by the epithelium (Thiersch and Remak). This synthesis becomes possible through the specific rearrangement of cells. Such rearrangements may result from developmental defects or through the action of stimulating substances. In the body not only quality but quantity is an absolute factor. *Systemic life* and *elemental life* are not comparable. The cells do not age. Differentiation is not an age phenomenon. The law of the conservation of energy holds for the body like it holds for all natural phenomena. Advancement in biology is to be made through the development of methods for the study of the mechanics of these systems. Morphology and chemistry must be supplemented by these methods. All parts of these machines are not visible. Rhythmical heart-muscle contraction is not a specific chemical reaction. It is peculiar to a large number of animals. These animals differ chemically from each other. A watch is a watch whether it is made of gold or silver.

The heart contracts as the result of a special arrangement of its parts and the specific dynamic properties of these parts, and not through the specific chemical constitution of the particular protein which compose its parts.

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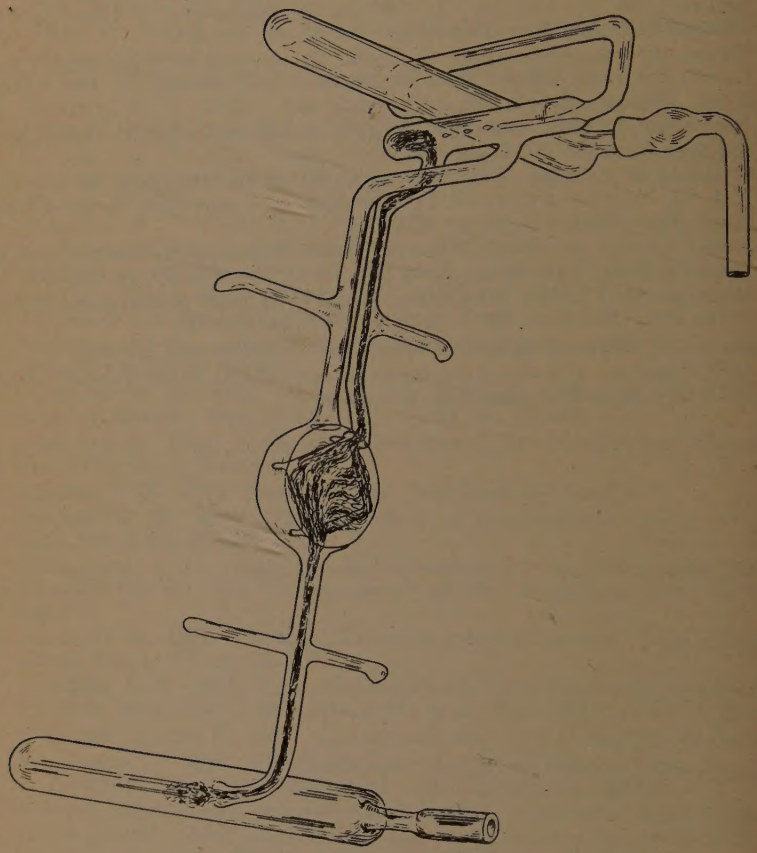
PLATE LII.



The "wick culture" chamber.